Neurotrophic Factors and Amyotrophic Lateral Sclerosis

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Abstract
The cause of motor neuron death in amyotrophic lateral sclerosis (ALS) remains a mystery. Initial implications of neurotrophic factor impairment involved in disease progression causing selective motor neuron death were brought forward in the late 1980s. These implications were based on several in vitro studies of motor neuron cultures in which a near to complete rescue of axotomized neonatal motor neurons in the presence of supplementary neurotrophic factors were revealed. These findings paved the way for extensive investigations in experimental animal models of ALS. Neurotrophic factor administration in rodent ALS models demonstrated a remarkable effect on survival of degenerating motor neurons and rescue of axotomized motor neurons, both in vivo and in vitro. In the absence of efficient therapy for ALS, some of these promising neurotrophic factors have been administered to groups of ALS patients, as they appeared available for clinical trials. Up to date, none of tested factors has lived up to expectations, altering the outcome of the disease. This review summarizes current findings on neurotrophic factor expression in ALS tissue and these factors’ potential/debatable clinical relevance to ALS and the treatment of ALS. It also discusses possible interventions improving clinical trial design to obtain efficacy of neurotrophic factor treatment in patients suffering from ALS.

Introduction
With a yearly incidence of 1–2 cases in 100,000 and a prevalence of approximately 4–9 in 100,000 individuals, amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease in adults [1–3]. The hallmark of this devastating neurodegenerative disorder is a preferential loss of upper motor neurons of the motor cortex and lower motor neurons of the brain stem and the spinal cord, leading to muscle deterioration, paralysis and ultimately death. No curative treatment is available for this rapidly progressing disease and the life expectancy of ALS patients after diagnosis is usually only 1–5 years. The etiology behind sporadic ALS is to date not known. Impaired neurotrophic factor production, release, and/or uptake however have been proposed as direct or indirect cause for the motor neuron loss observed in ALS. This proposal originated from several studies, demonstrating apparent neuroprotective effects after administration of tro-
Neurotrophic factors to rodent motor neurons under various in vitro and in vivo conditions [4–8]. These initial findings prompted extensive investigations on neurotrophic factor interaction in neuronal cell culture systems and in experimental animal models of ALS. Moreover, large-scale clinical trials applying these factors on groups of ALS patients were quickly initiated. Despite the enormous success neurotrophic factors demonstrated in rescuing motor neurons both in experimental animal models and in neuronal and organotypic cell cultures, only debatable therapeutic efficacy has been detected after administrating neurotrophic factors to patients suffering from ALS. These discouraging results of attempts at treating ALS are however not very surprising as our knowledge concerning optimal administration, transportation, uptake, half-life and therapeutic levels of neurotrophic factors in man is limited. The above-mentioned neuroprotective effects of exogenously administrated neurotrophic factors observed in cell cultures and in animal models of ALS, either relied on (a) measurements performed under standardized conditions (very different from the condition complexity of humans), (b) a primary shortage of neurotrophic factor(s), or (c) on primary motor neuron damage different from what is observed in patients with sporadic ALS. Excessive administration of exogenous trophic support to an organism that shows no obvious shortage of neurotrophic factors could potentially be harmful [9]. Neurotrophic factors’ ability to protect motor neurons has been reported to be dose-dependent [10, 11]. This protective effect declines once the optimal concentration of administrated trophic factor is exceeded. So far, no studies revealing a clear-cut impaired synthesis or decreased levels of neurotrophic factors and/or their corresponding receptors in tissue from patients living with ALS have been published. Instead, performed studies have reported on unaltered or increased levels of neurotrophic factors in spinal cord, muscle, brain, skin, cerebrospinal fluid (CSF) and serum from patients with ALS [12–19]. In addition, there is no clear evidence of a primary deficiency of neurotrophic factors causing ALS. Hitherto, molecular mechanisms behind neurotrophic factor activity, mode of action and their possible influence in selective motor neuron degeneration remain poorly understood.

This review summarizes current findings on neurotrophic factor expression in different tissues from ALS patients, motor neuron protection by neurotrophic factors in ALS animal models and in vitro cultures and these factors potential/debatable clinical relevance to ALS and the treatment of ALS.

Selective Motor Neuron Susceptibility and Trophic Support

ALS is primarily a disease affecting the motor neuron system. Susceptible neuronal populations in ALS are the upper and lower motor neurons, transmitting cortical signals to the peripheral muscles. These vulnerable sets of cells dying in ALS are generated in excess during embryonic development. Their initial survival depends on their own ability to make contact with their target tissue (the muscle) to avoid undergoing prenatal physiological motor neuron cell death [20–22]. This target-derived neuron-muscle contact and subsequent neuronal survival is guided and regulated by secreted neurotrophic factors [23]. Despite the prerequisite of neurotrophic factor guidance for the development and survival of motor neurons, studies in mice have demonstrated a normal motor neuron development and survival in the absence of certain neurotrophic factors [24–28]. Based on these studies relying on single gene mutants, the ensuing unaffected motor neuron development could be explained by pleiotropy and redundancy found among these neurotrophic factors [29–31]. However, certain neurotrophic factors are only readily expressed postnatal, indicating a regulatory and supportive function of these factors on motor neurons in adults [29, 32, 33]. Indeed, studies have shown that motor neuron depend on trophic support throughout life [34]. Because a selective motor neuron death is observed in ALS, a crucial question that remains to be countered is whether alterations in levels of neurotrophic factor accessible to the motor neurons precedes the onset of the disease or whether it is an unspecific response to present motor neuron damage.

Neurotrophic Factors, Related Proteins and Growth Factors

Neurotrophic factors are endogenous signaling proteins, promoting survival and well-being of specific populations of neurons as well as stimulating neuronal population differentiation. The neurotrophic factors are generally divided into three subgroups: neurotrophins, the glial cell-line-derived neurotrophic factor (GDNF) receptor ligands, and the neuropaotic cytokines. There is a continuously increasing number of neurotrophic factors being considered for clinical trials in ALS after demonstrating motor neuron protection in experimental models of ALS. Neurotrophic factors and related proteins which have revealed an ability to protect motor neurons are: ciliary...
neurotrophic factor [5], neurotrophin-3 [7], neurotrophin-4/5 [7], brain-derived neurotrophic factor [8], insulin-like growth factor [35], fibroblast growth factor [36], hepatocyte growth factor [37], interleukin-6 [38], leukemia inhibitory factor [39], cardiotrophin-1 [40], GDNF [6], neurturin [41], pigment epithelium-derived factor [42] and vascular endothelial growth factor [43]. Neurotrophic factors, related proteins, and growth factors with motor neuron potentiation and their corresponding receptors are listed in table 1.

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<tr>
<th>Classification</th>
<th>Factor</th>
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<tr>
<td>Neurotrophin family</td>
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<td>NT-3</td>
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<td>NT-4/5</td>
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<td>IL-6</td>
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<td>GDNF family and related proteins</td>
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**Neurotrophic Factor Production, Release, and Transport**

Neurotrophic factors are constitutively produced and are considered paracrine, endocrine or autocrine. Paracrine neurotrophic support could potentially include secretion from neighboring cells such as glial cells, Schwann cells or capillary endothelial cells, muscle fibers, short-axon afferent neurons (excitatory and inhibitory interneurons and parasegmental neurons) and long-axon afferent neurons (suprasegmental neurons and dorsal root ganglia neurons) [44]. Endocrine trophic support, on the other hand, relies on delivery from distant cells, such as epidermal cells, via blood or CSF [45]. Autocrine support refers to motor neurons own ability to supply neurotrophic factors. Several studies have demonstrated target-derived retrograde transport of these factors from the periphery (muscle) to the spinal cord and the ventral horn motor neurons in particular [6, 8, 46–48]. Anterograde transport from the upper motor neurons to the lower motor neurons has also been reported [46, 49, 50]. The above-mentioned different modes and sites of action neurotrophic factors are thought to exert their trophic support through are outlined in figure 1.

The muscle fiber constitutes the single final target tissue for motor neuron signaling. Moreover, the neurodegeneration observed in ALS is highly selective, primary affecting motor neurons. It is tempting to propose an imbalance in neurotrophic factor production or release in muscle tissue, secondarily altering the accessibility of trophic support for the motor neurons at the motor neuron terminals. This hypothesis implicates a primary deficiency within the muscle resulting in selective damage to motor neurons. Generally, it has been postulated that failure of muscle, glial and/or Schwann cells to release sufficient amounts of neurotrophic factors that maintain and enhance the well-being of lower motor neurons might lead to a loss of this specific cell population. Lower motor neuron degeneration would in turn, according to this hypothesis, deprive the upper motor neuron cells of target released and retrograde transported neurotrophic factors. Several recent studies have demonstrated alterations in the levels of neurotrophic factor expression in ALS muscle tissue [13, 17, 19]. Interestingly, these studies reported on increased levels of trophic factors, rather suggesting motor neuron damage as a result of excessive neurotrophic support instead of a general shortage. Additionally, available neurotrophic factor receptor studies in ALS tissue, confirm a spared and intact receptor signaling capacity in this disease [14, 51–54]. These findings imply an intact retrograde transport of neurotrophic factors from the muscle to the motor neurons in the ventral spinal cord. Excessive levels of neurotrophic factors in muscle tissue would consequently lead to excessive neurotrophic factor levels in motor neurons. Measurement of GDNF in both affected and unaffected muscle tissue from an ALS patient revealed increased GDNF levels in affected muscle but levels comparable to control case tissue in unaffected tissue [13]. As there is no evidence of an alteration in neurotrophic factor levels prior to symptom onset, increased levels of neurotrophic factors in affected tissue in ALS patients might represent an unspecific secondary event. As neurotrophic factors are also produced by and released from glial cells, the increased levels of neurotrophic factors observed locally in the CSF could be a secondary event due to gliosis, which has been shown to occur both in ALS brain [55] and spinal cord [56]. Both astrocyte [57] and microglial cell [58] counts are increased...
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Neurotrophic Factors and Corresponding Receptors in ALS Studies

Neurotrophins

Nerve Growth Factor (NGF)

NGF belongs to the family of neurotrophins and as the first isolated neurotrophin, it was initially described in the 1950s [67–69]. NGF has not been found to exert any specific neurotrophic effects on motor neurons in vivo or in vitro and hence has not been extensively investigated in relation to ALS. Performed studies of NGF’s potential protective effect on motor neurons have turned out negative, either revealing no influence or a negative influence on the survival of motor neurons [48, 70]. In spinal cord motor neuron areas from ALS patients, no specific expression of NGF or its corresponding high affinity receptor trk A have been detected [51]. Motor cortex from patients suffering from ALS have revealed decreased levels of NGF compared to control brains [71]. In a recent study, increased levels of NGF in postmortem muscle tissue from patients with ALS was observed [16].

Brain-Derived Neurotrophic Factor (BDNF)

BDNF was the second member of the neurotrophin family to be discovered, and was initially isolated from pig brain [72]. BDNF has been shown to promote survival of developing neurons [7, 73] and has been reported to rescue developing [8, 74–76] and adult [77] motor neurons from axotomy-induced or naturally occurring cell death [78]. Recently, Kawamoto et al. [79] performed a study localizing BDNF in ALS spinal cord sections. They observed immunoreactivity for the BDNF protein in remaining motor neurons in ALS at a similar magnitude as seen in control cases. Both protein and mRNA expression of BDNF’s high affinity receptor trk B, have been shown to be elevated in ALS spinal cord [54]. Investigations in both muscle biopsies and CSF samples from...
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patients with ALS revealed unaltered levels compared to measurements in control tissue [12, 13, 17, 80]. These findings suggest BDNF not to play an active part in the motor neuron degeneration observed in ALS. This conclusion is further supported by clinical trials, in which subcutaneous BDNF administration to ALS patients generally did not alter the outcome of the disease [81]. One subgroup (patients with early respiratory impairment) benefiting from the BDNF administration was however found and this finding initiated further clinical trials with high-dose subcutaneous administration or intrathecal administration of BDNF [82]. In the latter study, 25 ALS patients were infused with BDNF for 12 weeks in a sequential dose-escalating manner, using an implanted pump. Due to the small number of patients engaged and the study design no conclusions regarding the efficacy of the treatment could be drawn. No major adverse effects after administration of BDNF were however reported [83].

Neurotrophin 3 (NT-3)
The neurotrophin family member which is most abundantly expressed skeletal muscle, NT-3, has also been reported to have an impact on developing motor neurons [7]. NT-3 is expressed in developing motor neurons [84] and has been shown to act as a potent survival factor for upper motor neurons [85]. In postmortem ALS brain material, Duberley et al. [86] did not detect any difference in the number or intensity of neurons immunostained for NT-3 compared to control brain tissue. Another study from Duberley et al. [87] revealed a decreased NT-3 expression and unaltered trk C expression in ALS spinal cord tissue in comparison to equivalent control tissue. A recent study in postmortem ALS muscle tissue showed increased NT-3 expression, both at the mRNA and protein level [16].

Neurotrophin-4/5 (NT-4/5)
NT-4/5 was initially isolated from *Xenopus laevis* and was shown to reveal functional similarities with BDNF [88]. NT-4/5 also shares the high affinity receptor, trk B, with BDNF. NT-4/5 promotes survival of [73] and enhances acetyltransferase activity [7] in cultured rat motor neurons. NT-4/5 has not been extensively investigated in tissues from patients with ALS. A recent study however reported on increased mRNA levels of NT-4/5 in muscle tissue from ALS patients. This increase was enhanced during disease progression [16]. The number and intensity of NT-4/5 immunostained ALS cortical neurons in brain sections were unaltered when compared to control brain tissue [86].

Neuropoietic Cytokines

Ciliary Neurotrophic Factor (CNTF)
CNTF is a cytoplasmic protein belonging to the cytokine family. It was the first growth factor discovered to promote survival of lower motor neurons [4] and has demonstrated efficacy in rescuing a variety of experimental animal models presenting motor neuron degeneration [4, 89–92]. CNTF was also the first neurotrophic factor to be administered in clinical trials for ALS patients [9, 93]. Two large-scale clinical trials with subcutaneous CNTF administration in patients with ALS were conducted in the 1990s. No significant differences between the treatment groups and the placebo groups in either trial for any of the measured parameters or survival could be observed. Instead, dose-dependent undesired side effects such as febrile response were debilitating. Due to these adverse effects, CNTF is no longer being considered as a potential candidate drug in the treatment of ALS or other types of motor neuron diseases. Measurements of CNTF and its corresponding receptor, CNTFR-α, in spinal cord and motor cortex from patients affected by ALS revealed no impairment but instead increased levels of CNTF and CNTFR-α were detected [94, 95]. Elevated levels of CNTF have also been measured in skin biopsies from patients with ALS [96].

Leukemia Inhibitory Factor (LIF)
LIF was initially described as a glycoprotein able to enforce differentiation of myeloid leukemic cells [97]. LIF was later shown to promote survival of rat embryonic motor neurons [98] and to rescue axotomized motor neurons from newborn rats [99]. Investigations of LIF expression in ALS tissue are scarce, but ALS skin biopsies express increased levels of LIF compared to skin biopsies taken from patients with other neurological diseases [100]. This increase in LIF expression observed correlates to disease progression.

Cardiotrophin-1 (CT-1)
CT-1 is a muscle-derived cytokine, which is highly expressed in embryonic skeletal muscle and secreted by myotubes. It promotes the survival of cultured embryonic mouse and rat motor neurons [101], protects axotomized neonatal motor neurons [40]. CT-1 deficiency in embryonic mice results in partial motor neuron loss [101]. Both motor neuron cultures and axotomized motor neurons benefit from exogenous CT-1 administration [40]. CT-1 has yet not been investigated in ALS tissue.
Interleukin-6 (IL-6)

IL-6 is a cytokine usually derived from T cells. The factor can however be produced by other cells including astrocytes and microglial cells in the CNS. Conflicting results on levels of IL-6 in CSF from ALS patients have been reported. Sekizawa et al. [102] detected elevated IL-6 levels in ALS CSF, whereas Krieger et al. [103] did not find any significant difference when comparing the findings in ALS CSF and control CSF. Increased levels of IL-6 has also been observed in both serum and skin biopsies from ALS patients [104].

GDNF Family of Related Proteins

GDNF

GDNF, a distant member of the transforming growth factor-β (TGF-β) super family, was originally isolated as a factor from glial cell conditioned medium that promotes the survival and phenotype of ventral dopaminergic neurons [6]. GDNF has demonstrated great potency in supporting motor neuron survival in experimental models [6, 105]. Compared to the neurotrophins, GDNF reveals a 100-fold greater efficacy in rescuing spinal motor neurons [106]. In vivo, GDNF prevents programmed cell death and atrophy of motor neurons during axotomy-induced degeneration of motor neurons [47]. Increased levels of GDNF mRNA in postmortem spinal cord tissue [107] and GDNF protein in CSF [12] have been demonstrated in ALS patients in comparison to controls. Increased mRNA levels of GDNF expression has also been detected in muscle biopsies from patients with ALS [6,13,19]. Immunostaining for one of GDNF’s receptors, the Ret receptor, showed persistent expression of the Ret protein in remaining motor neurons in ALS spinal cord as compared to control cases [52]. Mitsuma et al. [53] also reported on a preserved GDNF receptor expression in ALS spinal cord, in an investigation comprising both mRNA and protein levels of GFR a-1, the specific receptor for GDNF. Ret mRNA and protein were exclusively expressed in motor neurons, whereas GFR a-1 was expressed in both motor neurons and glial cells. A study carried out in muscle tissue from patients with ALS revealed an unaltered expression of GFR a-1 mRNA in comparison to controls [19]. The same group failed to detect Ret mRNA in muscle tissue from ALS patients and control cases.

Immunoglobulin Factor (IGF) Family

IGF-1

IGFs belong to a family of structurally and functionally related proteins that include insulin, IGF-1 and IGF-2. Several studies have implicated that particularly IGF-1, may exert trophic support for motor neurons, and consequently most of its clinical development has focused on the treatment of motor neuron diseases [82]. Immunoglobulin factor IGF-1 has been suggested to support motor neurons [108] in pre-clinical studies based on efficacy on motor neuron survival in vitro [35, 109]. Initial clinical trials administrating IGF-1 subcutaneously to patients affected with ALS revealed a slight slowed progression of the disease [110, 111]. Larger trials however, failed to reproduce previous findings and no significant improvement in comparison to the placebo group was observed [112]. IGF-1 receptor immunoreactivity and levels of IGF-1 have been shown to either be unaltered [113, 114] or increased in ALS spinal cord [51, 113, 115] in comparison to control tissue analyses. These findings support the negative outcome of the clinical trials, where no benefit after administration of the factor was observed. Serum levels of IGF-1 among patients affected by ALS did not differ compared to serum levels of control cases [116].

Fibroblast Growth Factor (FGF) Family

FGF

FGF is a neurotrophic factor abundantly found in spinal cord and brain tissue in rat [117–119] and human [120]. FGF prevents death of spinal cord motor neurons in rats after experimental spinal cord injury [121, 122]. Interestingly, FGF has been detected in cholinergic motor neurons [117], which are affected in ALS. In fact, FGF has been localized to ventral horn cells in the spinal cord of ALS patients [114, 123]. Kage et al. [123] found decreased mRNA levels of FGF in spinal cord from ALS patients in comparison to controls. They also reported that 95.9% of cholinergic neurons in the ventral horn expressed FGF, at the same time as they detected a reduction in number of cholinergic neurons in the ventral horn spinal cord from patients with ALS. Another study reported on unaltered FGF receptor expression in ALS spinal cord compared to controls [114]. Recently, increased levels of FGF were detected both in CSF and serum from patients suffering from ALS in comparison to control cases [124].
Scatter Factor

Hepatocyte Growth Factor (HGF)

HGF is a secreted cytokine with neurotrophic activity [125, 126]. HGF has been demonstrated to protect and support motor neurons [37, 127] and was recently shown to be moderately up-regulated in CSF from patients with ALS in comparison to control CSF [14]. The corresponding c-Met receptor was unaffected in this patient group, suggesting an unaltered signaling ability [14]. Kato et al. [128] reported on similar or increased signaling intensity of both HGF and c-Met in ALS spinal cord motor neurons compared to control cases and c-Met induction in ALS spinal cord.

Serpin Family

Pigment Epithelium-Derived Factor (PEDF)

PEDF, a recently defined trophic factor, is a glycoprotein belonging to the serine protease inhibitor (serpin) family. PEDF has been shown to exert neuroprotection of motor neurons [42, 129]. Studies in ALS CSF revealed a significant elevation of this factor at the protein level in comparison both to CSF from other neurological patients and from patients with other neurodegenerative conditions [15] suggesting PEDF as a selective trophic factor for ALS.

Vascular Endothelial Growth Factor (VEGF) Family

VEGF

VEGF has long been recognized as a crucial factor in controlling the growth and permeability of blood vessels. It was recently hypothesized as a potential trophic factor for motor neurons. Oosthuyse et al. [43] discovered progressive motor neuron degeneration development in transgenic mice with a deletion in the hypoxia-response element of the Vegf promoter, ensuing in reduced hypoxic VEGF expression in the spinal cord of these mice. The pathogenesis of these transgenic mice is not fully understood but could in part be explained by a longstanding mild ischemia. This ischemia would result from a reduced neural vascular perfusion in these mice. In the same investigation, VEGF was also shown to protect rodent motor neurons in culture from cell death induced by serum withdrawal, hypoxia, or hypoglycemia. Further, VEGF was demonstrated to rescue motor neurons from axotomy-induced cell death in vitro, acting like a typical neurotrophic factor for this neuronal population [43]. VEGF has also been shown to be abundantly expressed in cultured spinal glial cells. No mutations in VEGF resulting in gene inactivation have been linked to human diseases so far. This is most likely due to the fact that the absence of even a single VEGF allele is embryonic lethal. Impaired hypoxic regulation of VEGF was recently shown to constitute a risk factor for ischemic heart disease, indicating that abnormal VEGF regulation, and not function, may predispose to pathological disorders. Recently, it was shown that a specific haplotype in the Vegf promoter sequence in a subgroup of ALS patients correlates with impaired VEGF plasma levels [130]. Attempts at measuring possible altered levels of VEGF in CSF from patients with ALS have so far been unsuccessful due to detection limitations in commercial ELISA kits [131]. Increased levels of VEGF have been reported in ALS serum in comparison to control serum. In the same study, VEGF levels in spinal cord from patients with ALS were shown to be unaltered compared to levels in control spinal cord tissue [18].

SR57746A (Xaliproden)

SR57746A, a small non-peptide compound, is thought to mimic neurotrophic factor activity or to exhibit neurotrophic activity through an induction of the natural biosynthesis of endogenous neurotrophic factors. The substance has revealed efficacy in mouse models of motor degeneration [132–134] and has also been administrated orally in a phase II clinical trial in treatment of ALS. The initial clinical trial reported a significant decrease in the rate of functional decline over the 8-month period administered but the two large-scale phase III follow-up trials, testing the compound administered over an 18-month period, were not able to reproduce the initial findings. Hence, SR57746A is no longer a candidate in the treatment attempts of ALS.

Experimental Animal and in vitro Models of ALS

Several experimental animal models of ALS have been presented over the past decades [for review, see 135]. Some of them develop spontaneous motor neuron degeneration due to genetic defects of autosomal recessive or dominant inheritance (i.e. pmn mouse, wobbler mouse, nmd mouse, mnd mouse and wasted mouse), while others rely on transgenic, knock out or deletion techniques (i.e. SOD1 mouse/rat, mice overexpressing neurofilament and...
Several of these animal models have benefited from subcutaneous [38, 90, 90, 136–140], intramuscular [141–143] or intraperitoneal [144, 145] injections of different neurotrophic factors. These favorable effects observed in animal models have relied on in vivo read-out parameters such as grip strength and weight change observations but also on in vitro parameters such as estimation of axonal degeneration and neuronal loss. Slowed disease onset and/or progression have been observed after administration of BDNF [137, 143], CNTF [92], LIF [144] or GDNF [142, 146] to these animals. Several neuropoietic cytokines (CNTF, LIF, IL-6) have proven to protect motor neurons from axotomy-induced death in a neonatal murine motor neuron degeneration model [38].

The, at present, only available agent used for treatment of ALS, riluzole, is an anti-excitotoxic substance that has been demonstrated to simulate the synthesis of BDNF and GDNF in cultured mouse astrocytes [147]. In vitro studies using organotypic spinal cord slice cultures or motor neuron cultures have also been performed. Corse et al. [10] tested the potential neuroprotective effect of neurotrophic factors in a rat organotypic spinal cord slice model of neurodegeneration caused by glutamate transport inhibition. Their data demonstrate protective effects using IGF-1, GDNF, and NT4/5, whereas BDNF, CNTF, and NT-3 could not prevent motor neuron death. In the same rat model, Bilak et al. [148] were later able to reveal a decreased motor neuron cell death after addition of GDNF and/or IGF-1. They also demonstrated an increased cell survival potentiation of GDNF when combining the treatment of GDNF with IGF-1. Such additive effects when applying more than one neurotrophic factor has been described in several reports [89, 91, 149]. This indicates that the regulation of motor neuron survival is a highly complex process that is influenced by more than one neurotrophic factor.

**Conclusion and Discussion**

Every attempt at treating ALS by application of neurotrophic factors has so far turned out a failure. Despite these discouraging therapy outcomes, several options on improvement of trophic factor therapy in ALS deserve to be further investigated.

**Pre-Clinical Testing**

Once shortage of neurotrophic factor(s) has been determined in relevant tissue from patients with ALS in comparison to control tissue, this neurotrophic factor might be considered for clinical trials. A reasonable initial approach could include pre-clinical testing by means of local (non-systemic) injections. Instead of rapidly setting off very expensive, time-consuming, large-scale multicenter trials, it might be worthwhile to test the efficacy of a neurotrophic factor on a small group of ALS patients. Injection of this factor directly to the site of normal secretion, at the motor endplate, by unilateral frequent (daily) local injection into one defined affected or unaffected peripheral muscle and use the opposite muscle for placebo injection could provide valuable information on the potential protective effect of the neurotrophic factor assigned to be tested in clinical trials. In vivo gel electroporation of GDNF into skeletal muscle of SOD1 mice has provided evidence of the feasibility of such an approach and the relative low cost [150].

**Optimizing Administration**

To date, very little attention has been devoted to optimizing the administration of neurotrophic factors in the treatment of ALS. Performed clinical trials have relied on oral, subcutaneous or intrathecal administration of applied neurotrophic factors. Many neurotrophic factors are locally produced in cells that are in direct contact with the motor neurons. Several studies have detected normal signaling ability for trophic factor receptors. Since the half-life of several available trophic factors is very short, systemic administration might not provide optimal trophic factor levels accessible to the motor neurons. Administrating direct at the site of secretion might be one way to circumvent this predicament. Another way to obtain efficacy would be to allow the trophic factor passage through the blood-brain barrier. This requires the design of molecules of a size and hydrophobic character that will allow passage through the blood-brain barrier or the coupling of the neurotrophic factor to a transporter molecule that can pass the blood-brain barrier. Either technique will certainly provide a frame for future administration improvements. Albeck et al. [151] have presented a successful non-invasive transport system for GDNF using antibodies against transferring receptors in the blood-brain barrier. A recently developed technique relying on transduction of recombinant adeno-associated virus vectors [152] transporting and delivering factors from the site of injection in the muscle to the motor neuron somata in a retrograde fashion, has provided promising data when treating SOD1 mice with IGF-1 [153]. With this administration technique, not only animals receiving IGF-1 before disease onset presented an increased survival, but also animals that were provided initial treatment at time...
of symptom onset lived longer than untreated animals. At this point, neurotrophic factor delivery via adeno-associated virus vectors has not been fully evaluated with respect to longstanding tolerance and efficacy. Further studies addressing this concern needs to be undertaken and delivery to patients suffering from ALS will likely request additional refinement and modification of the system. Further development of small molecules, such as SR57746A, that either mimic or potentiate the expression and activity of naturally occurring neurotrophic factors might also be helpful for improved treatment of ALS. Clinical trials administrating neurotrophic factors need to be carefully monitored to assure a secure, rapid, and adequate uptake of applied trophic factor. If not, desired doses of active substance might never be achieved. Moreover, we need to reach a better understanding of the action of these neurotrophic factors outside the nervous system to limit potential and evident adverse effects to a minimum in correlation to increasing doses.

**Novel Neurotrophic Factors**

With a continuously growing list of trophic factors described, it is possible that the ultimate trophic factor(s) for motor neurons remains to be discovered. Other newly characterized members of the GDNF family of trophic factors, i.e. artemin [154], persephin [155], and neurturin [156] or related factors, might offer a more directed and specific support of motor neurons. None of these factors has appeared in the literature yet as tested positive as drugs for motor neuron rescue in ALS. Further, no studies have been brought forward, in which the levels of either of these factors have been measured in ALS tissue.

**Neurotrophic Factor Cocktail Treatment**

The most recent experiments in animal models of ALS focused on additive effects on motor neuron protection when treating the animals with a combination of different neurotrophic factors. Providing a cocktail of neurotrophic factors might turn out more effective in the treatment of patients suffering from ALS. This tempting approach however raises questions such as which neurotrophic factors to mix, what concentrations to use, and during which phases of the disease progression to distribute the different neurotrophic factors.

**Animal Models of ALS**

Bearing the above-mentioned reservations in mind, the initial optimism which neurotrophic factor treatment for ALS was given must be re-evaluated. An animal model strictly resembling ALS in its course, with upper and lower motor neuron degeneration in adulthood, is mandatory if we want to draw decisive conclusions from neurotrophic factor studies in animals for improved treatment of ALS patients. Models where immature motor neurons are treated cannot fully be compared to the human condition. A comparative study in man and rat spinal cord tissue revealed extensive species difference which further points towards the difficulty in drawing conclusions from rodent models [114]. As long as we rely on present ALS animal models, it is first necessary to understand the underlying mechanisms, which provide neurotrophic factor protection in current models, before adapting the successful treatments to the human clinical syndrome. Moreover, many studies in ALS animal models demonstrating a clear neuroprotection are based on neurotrophic factor treatment initiated before symptom onset. At present, patients with ALS neither receive neurotrophic factor treatment prior to symptom onset, nor at symptom onset. Instead, most patients only receive treatment once diagnosis has been confirmed. The average time span between symptom onset and diagnosis, allowing the possibility of treatment initiation, is approximately 13 months [157].

As reviewed in the present article, no evidence of clear neurotrophic factor impairment in patients with ALS is available. Taken together, the above-listed findings suggest that motor neurons in ALS patients may receive sufficient neurotrophic factor supply and that there is no demand for further support of these factors from surrounding tissues. Available data instead point towards a possible toxic effect on motor neurons due to excessive levels of neurotrophic factors in this disease. It needs to be stressed that as long as we have not detected decreased mRNA or protein levels of any neurotrophic factor or down-regulation of corresponding receptors in ALS patients, the likelihood of success when applying these agents on patients suffering from ALS is small.

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